

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **09/328,975**

Confirmation No. 7574

Applicants : **Jon A. Wolff, et al.**

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Art Unit : **1635**

Examiner : **Schnizer, Richard**

Docket No. : **Mirus009**

For: **Charge Reversal of Polyion Complexes**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

DECLARATION UNDER 37 C.F.R. ' 1.132

Dear Examiner:

I, Vladimir Trubetskoy, hereby declare as follows:

1. I am an inventor of the captioned application.
2. The attached notebook pages (nos. 120-122) contain the experiment and data that is described in the specification in example 6 starting on page 26.
3. The abbreviation used throughout this notebook for polyaspartic acid was pAsp (see page 22 of the notebook). The abbreviation for polyacrylic acid used throughout the notebook was pAA (see page 111 of the notebook).
4. The polymer used in the experiment disclosed in the specification in example 6 was polyacrylic acid.
5. The abbreviation listed in the specification on page 22 line 27 for polyaspartic acid is a typographical error. The correct abbreviation for polyaspartic acid is found on page 6 line 26 and page 20 line 4 of the specification. Also note that the abbreviation pMAA, for

polymethylacrylic acid (see page 6 line 26 and page 20 line 4 and page 111 of the notebook),
is consistent with PAA being the abbreviation for polyacrylic acid.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Dr. Vladimir S. Trubetskoy

4/18/05
Date

VIAL	43	237 LR > Liver
#3	44	208 MC
SplL	45	311 - Spleen
	46	151 - Lung
	47	128 - heart
	48	184 - kidney
	49	346 - tail
SAMPLE		RLU

VIAL	1	221 LR > Liver
#3	2	234 MC
SplL	3	173 Spleen
	4	156 Lung
	5	141 heart
	6	156 kidney
	7	395 tail

VIAL	8	216 LR
#4	9	142 MC
PEG XL	10	163 Spleen
	11	187 Lung
	12	177 heart
	13	413 kidney
	14	221 tail
VIAL	15	185 LR > LIVER
#4	16	198 MC
PEG XL	17	161 Spleen
	18	162 heart
	19	217 kidney
	20	206 kidney
	21	217 Tail

In general, though all numbers are very low
non XL samples worked better (liver 15 times, tail)
So XL-particles are too stable

DNA/PLL34/PEG-SPLC particles did not aggregate upon
addition of salt. results in highest tail activity

2/10/99

Synthesis of PEG-pAsp conjugates

* Polyaspartic acid (pAsp) - $M_w = 36 \text{ kDa}$ decondensing polyanion

Idea - to modify pAsp with increasing #
of PEG chains to modify its ability to
decondense DNA from its DNA/PLL complex

% of modification will establish the rate of
DNA release

V. Trubetsky 2.10.99

Pl-DNA decondensation with different polyacrylates

Polyacrylates: pMAA 9 μ Dg
pAA 30 μ Dg
pPAA ? μ Dg Davis

DNA* 10 μ was condensed with 12 μ PLL 3 μ
in 5 mM KESFES pH 7.5.

No.	FI	[ug/ml]	10 μ of each polyacrylate were added to decondense
1	-0.016	0.0118	
2	56.588	0.4026 DNA	
3	8.452	0.0703 DNA/PLL	
4	46.071	0.3300 10 μ pAA	
5	56.180	0.3998	
6	48.614	0.3476 DNA	
7	8.687	0.0719 DNA/PLL	
8	9.190	0.0754 10	
9	8.740	0.0723 20	
10	8.083	0.0678 30	
11	7.983	0.0671 40	
12	7.660	0.0648 50 of pPAA	
13	7.405	0.0631 60 μ	

↳ this does not work

4.29 ~~99~~ Cell-binding results of experiment on p. 110.

Polyanions alone did not work - no cell binding
but probably concentration was too low

Particles

(1) SPL 210 vs SPL 460 no XL - particles definitely disassemble on cell surface, SPL almost invisible only DNA (may be intensity), no internalization

460 particles are more stable than 210
(note colocalization)

(2) XL particles bind less than no-XL but with more colocalization (expected)

(3) noXL-460 one can see some SPL binding

in vivo DNA/PEI/pAA

Samples recharged ~ 4L pAA before and after neutrality

DNA/PEI/pAA

per animal: (1) 50g / 100g / 40g
 (2) — 11 — 50g + } in 0.25 ml
 (3) — 11 — 60g ++ } cloudy 1.6
 (4) — 11 — 70g }

Results of in vitro binding exp. from So and KirkBNL
Hela

Kirk: all polyanions bind very weakly more with RGD less with PEG and SPCL

Particles bind more strongly with RGD more PEG less much more with SPCL

So: general binding less than usual (XL!) with no difference from amount of SPCL.

5/6/99

Work with cyclic RGD peptideRGD peptide (Mirus) G-G-C-R-G-D-M-F-G-C $M_w = 1000$

18 mg diss. in 1 ml MeOH + 15X of conc. HCl

add 400 of I_2 solution in MeOH (50c)

- in 5X increments → until yellow color stays

Precipitate with $2H_2O$

8 mg of peptide + 27.2 mg of Fmoc-PEG-NHS

in 0.7 ml of 0.1M HEPES pH 8.0, 0.5M NaCl

VOLUME

disap. of M_{12} groups during reaction

S# 420.0

1 0.0003

2 0.0060

3 0.6759

after

before react

10x / 0.5 ml of borax

after reaction - dial. ag. H_2O in 3.5 kDa
MWCO overnight, $4^{\circ}C$.

folate particles for So:
precip. from p. 114

- (1) Rh DNA / PCL34 / Cy5 SPCL210 - PEG folate 50 / 70 / 250
(2) — 11 — SPCL (5%) - PEG — 11 —
(3) — 11 — Cy5 SPCL210 50 / 70 / 150

activated with 100 / 200 EDC / S-NHS.

Results of 1/2 low pressure injections from p. 120

#1			#2		
	1	1548 LR	19	3954 LR	
VIAL	2	1211 MC	20	6594 MC	Vial 2
Animal	3	1093 Spleen	21	2461 Spleen	(210)
	4	166931 lung	22	10074 lung	Animal 5
	5	2722 Heart	23	211 Heart	
	6	381 Kid.	24	136 Kidney	50/100/50
	7	4041 LR	25	1374 LR	
VIAL	8	1986 MC	26	2538 MC	
Animal	9	6412 Spleen	27	2293 Spleen	Animal 6
2	10	197443 lung	28	16544 lung	Vial 2 (210)
50/100/40	11	1649 Heart	29	196 Heart	
	12	916 kidney	30	178 kidney	

one animal died 1/3

VIAL	13	2951 LR	one animal
2 (210)	14	2290 MC	died (1/4)
Animal	15	1022 Spleen	
LR	16	1557 lung	
	17	66 Heart	
	18	12144 kidney	

31 11604 LR #3
32 5822 MC 50/100/60
33 225 Spleen Vial 3
34 333 lung 7 animal
35 76 Heart
36 91 kidney
37 21517 LR
38 22765 MC Vial 3
39 4404 Spleen Broad #9
40 290 lung
41 57 Heart
42 82 kidney
43 12606 LR
44 12913 MC Vial 3
45 314 Spleen Broad #9
46 352 lung
47 95 Heart
48 67 kidney

Surv.

(0/3)

BKC

BKC

#4
50/100/70
49 254 LR
50 147 MC
51 556 Spleen
52 70368 lung
53 583 Heart
54 139 kidney
55 388 LR
56 587 MC
57 1507 Spleen
58 150514 lung
59 186 Heart
60 260 Spleen
61 429 LR
62 407 MC
63 1858 Spleen
64 266123 lung
65 3018 Heart
66 288 kidney

Surv.

(0/3)

Vial
#4
Animal
#10Animal
#11Animal
#12